Bone Marrow RNA Extraction

Lymphoprep

Company: Stem Cell Technologies
Catalog Number: 07801
Quantity per box: 500mL
Price: $103

Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum

Company: Stem Cell Technologies
Catalog Number: 07905
Quantity per box: 500mL
Price: $28.00

Cost per Sample: $8.38

Samples will be collected in an anticoagulant tube. Lymphoprep can be stored at room temperature, and stored away from light. Samples need to be processed immediately after aspiration.

Directions for Lymphoprep use:

1. Mix Lymphoprep thoroughly before use by inverting the bottle several times.
2. Add 1.5mL of Lymphoprep to 5mL tube
3. Dilute 1 mL bone marrow with 1mL of phosphate-buffered saline plus 2% fetal bovine serum (PBS+ 2% FBS; Catalog #07905) in a separate tube.
4. Layer bone marrow mixture on top of Lymphoprep in 5mL tube being careful to minimize mixing of blood marrow mixture with Lymphoprep
5. Centrifuge at 800 x *g* for 20 minutes at room temperature (15 - 25°C) with brake off. Remove and discard upper plasma layer without disturbing the plasma-Lymphoprep™ interface.
7. Remove and retain mononuclear cell layer at the plasma-Lymphoprep™ interface without disturbing erythrocyte/granulocyte pellet into a new 5mL tube.
8. Wash mononuclear cells once with 4mL phosphate-buffered saline plus 2% fetal bovine serum.
    a. centrifuge at 300 x g for 10 minutes
    b. aspirate the supernatant. Cell pellet will remain at the bottom.
9. Calculate average number of cells needed per sample: 3–4 x 10^6 cells. Maximum number of cells that can used per sample is 1 x 10^7.
10. Flick the cell pellet to loosen from the bottom of the tube, then disrupt the cells by adding 350 uL Buffer RLT Plus with β-ME if the number of cells are <5 x 10^6. Add 600uL of RLT Plus buffer with β-ME if the number of cells range from 5 x 10^6– 1 x 10^7 (GT will provide RLT Buffer Plus with β-ME). Vortex or pipet to mix. Mix thoroughly. Inefficient mixture can lead to a decrease in RNA yield.
    a. β-mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus is stable at room temperature (15–25ºC) for 1 month after addition of β-ME.
11. Pipet the vortexed lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed.
12. Each blood marrow aspirate needs to be extracted at the same time of day and in the same chronological order per animal.
    i.e. Monkey A has the first aspirate of the day at 10:00am. Each time thereafter, Animal A should always be the first aspirate of the day at that time so we do not see a batch effect in our results.
13. Have all samples labeled with date and time along with ID.
14. Samples can be stored in -80 freezer until RNA extraction.